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Assessment of SARS-CoV-2 in human semen—a cohort study

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Objective: To investigate the presence of viral RNA in human semen of patients with severe acute-respiratory syndrome coronavirus 2 (SARS-CoV-2) and to evaluate its presence and relevance in semen parameters.
Design: Pilot cohort study.
Setting: University hospital.
Patient(s): Thirty-four men were distributed as: 1) patients in convalescence (patients with confirmed SARS-CoV-2 infection in pharyngeal swab according to reverse-transcription polymerase chain reaction [RT-PCR] or antibodies); 2) negative control group (no antibodies); and 3) patients with an acute infection (detection of SARS-CoV-2 in pharyngeal swab).
Intervention: Semen and a blood sample were collected from each individual.
Main Outcome Measure(s): Analysis of semen quality according to the World Health Organization standards. Detection of SARS-CoV-2 by RT-PCR in the native semen sample and after density gradient preparation. Confirmation of immunoglobulin (Ig) A und IgG antibodies in the blood.
Result(s): Eighteen semen samples from recovered men were obtained 8–54 days after absence of symptoms, 14 from control subjects, and 2 from patients with an active COVID-19 infection. No RNA was detected by means of RT-PCR in the semen, including semen samples from two patients with an acute COVID-19 infection. Subjects with a moderate infection showed an impairment of sperm quality.
Conclusion(s): A mild COVID-19 infection is not likely to affect testis and epididymis function, whereas semen parameters did seem impaired after a moderate infection. SARS-CoV-2 RNA could not be detected in semen of recovered and acute COVID-19–positive men. This suggests no viral transmission during sexual contact and assisted reproductive techniques, although further data need to be obtained. (Fertil Steril® 2020;114:233-8. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: SARS-CoV-2, COVID-19, semen, infertility, ART

In December 2019, clusters of a novel type of pneumonia were reported in Wuhan City, Hubei Province, People's Republic of China (1), and defined by the World Health Organization (WHO) as coronavirus disease 2019 (COVID-19) in February 2020 (2). The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the causing viral pathogen for the subsequent pandemic (3). To constrain the worldwide outbreak of COVID-19, viral transmission pathways have been intensively studied. So far, it is known that the coronavirus is predominantly transmitted through respiratory droplets (4). In addition, viral RNA has been detected in various biological samples, such as feces, urine, and blood (5). SARS-CoV-2 seems to have a high-affinity binding capability to the angiotensin-converting enzyme 2 (ACE2) in human cells, which is expressed in multiple organ systems, including the testes (6). Although the testes are immunologically privileged in case of viremia, some viruses can cross the blood-testis barrier, causing local inflammation (7). The virus may persist after an acute infection, for example, human immunodeficiency virus (HIV), and can theoretically replicate within the male reproductive tract (8). Thus, viral RNA of primarily nonsexual transmitted diseases can be found in semen (9).

The presence of SARS-CoV-2 in the male reproductive tract may reduce male fertility through orchitis or spermatogonial stem cell infection and may have implications for sexual transmission and consequently for

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embryonic infection, miscarriage, and congenital disease (10). To thoroughly advise couples with acute desire for a child, information about the impact of COVID-19 on male reproductive function and viral seeding are needed.

The aims of the present study were to: 1) determine any possible implications of COVID-19 on male semen parameters; and 2) analyze the semen for any presence of SARS-CoV-2 RNA in recovered men and men with an active COVID-19 infection.

MATERIALS AND METHODS

This prospective cohort study enrolled 34 men from April 24 to May 6, 2020, at the interdisciplinary reproductive unit of the University Hospital in Duesseldorf, Germany. Local institutional review board (Heinrich Heine University Duesseldorf) approval (study no. 2020-938) was obtained before study initiation, and written informed consent was given by each participating individual. To confirm the systemic presence or absence of SARS-CoV-2, a blood serum sample (BD Vacutainer) was collected for analysis of antibodies. Men with a positive swab result (ESwab collection kit; Copan) or positive Immunoglobulin (Ig) A and IgG antibodies were considered to be positive for COVID-19. Patients were classified as having a mild COVID-19 infection when home care was possible. Moderate COVID-19 was defined for patients requiring hospitalization with up to 6 L oxygen supplied to achieve >92% peripheral oxygenation. The control group consisted of healthy volunteers with no reported andrologic pathology.

Sperm Sampling and Preparation

A semen sample of each participant was obtained by means of masturbation and ejaculation directly into noncytotoxic sterile containers. Freshly collected semen was liquefied for 30–60 min at room temperature and processed within 1 hour of ejaculation for analysis of sperm characteristics according to the criteria published by WHO. Sperm morphology was not assessed owing to safety concerns. Samples were homogenized, and 500 μL was transferred to the tube for viral testing of the native sample.

To prepare the semen sample for the viral testing, the remaining semen was prepared in a two-step washing process modified according to the center's standard procedure for men with HIV or hepatitis C infections. First, the semen was counted and filtered through a 30°C prewarmed 90%/45% colloidal silica density gradient at 1.800 rpm for 20 min (SpermFilter; Gynotec) and prepared with GM 501 SpermAir (SPA) sperm processing medium (Gynemed). Second, the pellet was washed in 3 mL prewarmed SPA at 2.300 rpm for 10 min and the resulting pellet resuspended in 500 μL SPA, counted, and transferred to the viral testing tube of the processed sperm.

Detection of SARS-CoV-2 in Semen

The native and processed sperm sample was centrifuged for 1 min at 3.500 rpm. RNA extraction was performed from 200 μL supernate with the use of the EZ1 Virus Mini Kit v2 (Qiagen) following the manufacturer's instructions; 60 μL was eluted from the 200 μL starting material, and 5 μL of the eluate was tested by means of reverse-transcription polymerase chain reaction (RT-PCR) with the use of the TaqMan technique. A 113-base-pair amplicon in the E-gene of SARS-CoV-2 was amplified and detected, as previously described with minor modifications (11). RT-PCR was performed with the use of an ABI 7500 FAST sequence detector system (PE Applied Biosystems). The thermal protocol described was shortened to 40 cycles of 95°C. We used the LightMix Modular SARS and Wuhan CoV E-gene (cat. no. 53-0776-96) and the LightMix Modular EAV RNA Extraction Control. Moreover, we used the AgPath-ID One-Step RT-PCR Kit (Applied Biosystems cat. no. 4387391; DNA-standard plasmid pEX-A128-nCoV2019-E-gene).

SARS-CoV-2 Antibody Detection

We used a commercial anti–SARS-CoV-2 S1 IgG and IgA ELISA (IgG cat. no. EI 2606-9601G, IgA cat. no. EI 2606-9601 A; EuroImmun Medizinische Labordiagnostika) following the manufacturer's instructions; 500 μL serum was tested with the use of the fully automatic EuroImmun Analyzer I-2 P (EuroImmun Medizinische Labordiagnostika). This kit does not include IgM antibodies, which are not expected to be found in recovered individuals. According to the manufacturer (EuroImmun), the following sensitivity and specificity of the commercial anti–SARS-CoV-2 S1 IgG and IgA ELISA are indicated: IgG sensitivity increases from 30.3% <10 days after start of symptoms to ~94% after >21 days. IgG specificity is high, at ~99%. IgA sensitivity increases from 51.5% <10 days after start of symptoms to 100% after >21 days. IgA specificity also is high, at ~88%.

Statistical Analysis

Statistical analysis was performed with the use of SPSS 23 and Mann-Whitney U test. Two-sided P values $<.05$ were considered to be statistically significant.

RESULTS

The study population consisted of 18 men who were recovered from an infection with SARS-CoV-2 and a control group of 14 men who were not affected. Moreover, two subjects had an acute infection with SARS-CoV-2. The recovered participants were 42.2 ± 9.9 years old with body mass index (BMI) 25.6 ± 2.9 kg/m^2, and the control group were 33.4 ± 13.1 years old with and BMI 24.6 ± 2.6 kg/m^2 (no statistical differences). No study participant suffered from any preexisting illnesses, including hypertension and diabetes mellitus (Table 1).

Symptoms and Virology Testing

Seventeen of the 18 recovered participants described symptoms, mainly fever (10 out of 18), cough, headache, ague, muscle pain, body ache, dyspnea, and fatigue. Two participants had anosmia and loss of taste. One participant reported testicular discomfort.

Four participants with a moderate course of disease were hospitalized owing to high fever and dyspnea. None of them needed endotracheal intubation. However, two subjects

TABLE 1

Characteristics for male individuals and associated results of COVID-19 analysis in blood and semen samples.

Characteristic	Mild	Moderate	Control
Individuals, n	14	4	14
Age, y	42.7 ± 10.4	40.8 ± 8.7	33.4 ± 13.1
Body mass index, kg/m^2	25.0 ± 2.3	27.7 ± 4.1	24.6 ± 2.6
Smoker	2/14	0/4	2/14
Oropharyngeal swab positive	13/14	4/4	–
Antibodies positive (IgA or IgG)	13/14	4/4	0/14
IgA positive	13/14	4/4	0/14
IgG positive	12/14	4/4	0/14
SARS-CoV-2 in semen	0/14	0/4	0/14
COVID-19–related symptoms	13/14	4/4	–
Duration of symptoms, d	8.6 ± 9.1[a]	28.2 ± 16.2[a]	–
Testicular discomfort during infection	0/14	1/4	–
Hospitalization	0/14	4/4	–
Duration of hospitalization, d	-	9.2 ± 5.3	–
Time between positive oropharyngeal swab and semen collection, d	43.5 ± 6,2	47.0 ± 5,3	–
Time between end of symptoms and semen collection, d	34.9 ± 11.7	25.5 ± 8.3	–

Note: Data presented as mean ±standard deviation, unless specified otherwise. Statistical analysis according to Mann-Whitney *U* test for nonparametric distribution.
[a] $P < .05$.

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received antiretroviral therapy with lopinavir/ritonavir for 1 day in one case and for 3 days in the other case. The third subject was given hydroxychloroquine and moxifloxacin. Other medication taken by some participants was paracetamol.

The control group had not suffered from any symptoms related to COVID-19 in the previous 8 weeks.

Of the subjects with an acute SARS-CoV-2 infection, one had no symptoms, whereas the other presented with cough, headache, and ague. IgA and IgG antibodies were present in 17 of the 18 COVID-19–recovered men. One of the recovered individuals with confirmed SARS-CoV-2 RNA in the pharyngeal swab during the acute infection developed neither antibodies nor symptoms. Another recovered man with failed detection of SARS-CoV-2 RNA in the pharyngeal swab during the active infection presented IgA and IgG antibodies. He reported only a headache for 2 days. No antibodies could be detected in the serum of any of the control subjects (Table 1).

TABLE 2

Semen parameters for COVID-19–positive individuals and control subjects.

Semen parameter	Mild	Moderate	Control
Individuals, n	14	4	14
Sexual abstention, d	3.2 ± 1.1	2.5 ± 1.0	3.3 ± 1.9
Volume, mL	2.5 ± 1.0	1.4 ± 0.7	2.51 ± 1.1
Sperm concentration, 10^6/mL	95.9 ± 50.5[a]	16.2 ± 22.4[a,b]	89.5 ± 69.6[b]
Total no. of sperm per ejaculate, 10^6	243.7 ± 140.4[a]	11.9 ± 13.4[a,b]	233.1 ± 234.4[b]
Total no. of progressive motility, 10^6	125.3 ± 96.4[a]	2.4 ± 2.7[a,b]	102.1 ± 102.3[b]
Total no. of complete motility, 10^6	157.1 ± 120.8[a]	4.7 ± 5.5[a,b]	124.0 ± 124.9[b]
Total no. of immotile, 10^6	86.6 ± 66.5[a]	7.2 ± 9.4[a,b]	109.1 ± 121.0[b]
Leucocytes detected	11/14	3/4	14/14
Bacteria detected	8/14	3/4	9/14
Individuals, n	14	2 [c]	14
Sexual abstention, d	3.1 ± 1.1	3.0 ± 1.4	3.3 ± 1.9
Volume, mL	2.5 ± 1.0	1.1 ± 0.9	2.51 ± 1.1
Sperm concentration, 10^6/mL	95.9 ± 50.5[a]	32.0 ± 22.6[a,b]	89.5 ± 69.6[b]
Progressive motility, %	46.1 ± 21.1	20.0 ± 0	42.1 ± 17.8
Complete motility, %	57.5 ± 24.1	42.5 ± 24.7	51.1 ± 18.1
Immotile, %	42.5 ± 24.1	57.5 ± 24.75	48.9 ± 18.1
Leucocytes detected	11/14	1/2	14/14
Bacteria detected	8/14	1/2	9/14

Note: Data presented as mean ±standard deviation, unless specified otherwise. Statistical analysis according to Mann-Whitney *U* test for nonparametric distribution. No statistically significant differences could be detected between patients with mild symptoms and control subjects.
[a] $P < .05$, mild vs. moderate.
[b] $P < .05$, moderate vs. control.
[c] Two patients with cryptozoospermia according to WHO classification were excluded from the analysis.

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TABLE 3

Semen parameters for COVID-19 positive individuals with and without fever during infection.

Semen parameter	Fever negative	Fever positive
Individuals, n	8	10
Sexual abstention, d	3.1 ± 1.0	3.1 ± 1.2
Volume, mL	2.8 ± 0.9[a]	1.8 ± 0.9[a]
Sperm concentration, 10^6/mL	100.9 ± 31.1[b]	60.0 ± 66.8[b]
Total no. of sperm per ejaculate, 10^6	283.6 ± 124.0[b]	119.0 ± 147.5[b]
Total no. of progressive motility, 10^6	142.0 ± 93.2	62.8 ± 93.8
Total no. of complete motility, 10^6	185.6 ± 122.1[a]	73.4 ± 106.3[a]
Total no. of immotile, 10^6	98.01 ± 67.6[a]	45.7 ± 60.6[a]
Leucocytes detected	5/8	9/10
Bacteria detected	4/8	7/10
Individuals, n	8	8
Sexual abstention, d	3.1 ± 1.0	3.4 ± 1.2
Volume, mL	2.8 ± 0.9[a]	1.7 ± 1.0[a]
Sperm concentration, 10^6/mL	100.9 ± 31.1	74.9 ± 66.9
Progressive motility, %	49.4 ± 19.7	36.3 ± 22.8
Complete motility, %	63.1 ± 22.7	48.1 ± 24.0
Immotile, %	36.9 ± 22.7	51.9 ± 24.0
Leucocytes detected	5/8	7/8
Bacteria detected	4/8	5/8

Note: Data presented as mean ± standard deviation, unless specified otherwise. Statistical analysis according to Mann-Whitney *U* test for nonparametric distribution.
[a] $P < .05$.
[b] Different by trend.

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Main Outcomes

SARS-CoV-2 RNA could be detected in semen samples from neither recovered nor acute infected subjects. The results of the sperm analysis are summarized in Table 2. Patients with a moderate infection have a statistically significant impairment of sperm quality (sperm concentration, total number of sperm per ejaculate, total number of progressive motility, total number of complete motility) compared with men recovered from a mild infection and the control group. We divided the individuals as fever positive versus fever negative regardless of their classification into mild and moderate and analyzed the semen accordingly as presented in Table 3. Although there were statistical significant differences regarding the volume, the complete motility, and the number of immotile sperms, the values were all still in the normal range (Table 3).

DISCUSSION

ACE2 is the cell entry receptor for SARS-CoV-2 and it is found not only in the respiratory system but also in the testis. This finding led to the hypothesis that the human testis, and therefore semen, is a target for a SARS-CoV-2 infection, which might increase the understanding of this rapidly spreading disease (12). Furthermore, the investigation of semen samples regarding the presence of SARS-CoV-2 RNA is highly important, because it has been shown for several different viruses that viremic patients can shed viruses into their semen (9). Moreover, there are viruses, such as the Zika virus, that can remain in the semen of symptom-free individuals for up to 12 months after recovery and thereby provide a risk for long-term transmission exposure (13). Our data suggest, however, that SARS-CoV-2 most likely does not shed into the semen, or at least not in the investigated time period after the end of symptoms of 32.7 days on average as presented in this study. This hypothesis is supported by a recent study showing a sparse expression of ACE2 and transmembrane serine protease 2 gene expression in men who had a median time of 31 days from the confirmation of diagnosis to the collection of semen and no presence of SARS-CoV-2 RNA in the investigated semen samples (14). The fact that only very low titers of SARS-CoV-2 have been detected so far in nonrespiratory sites such as feces and urine specimens (5, 15) also supports the hypothesis that SARS-CoV-2 shows only a minor risk of virus shedding into the semen. Nevertheless, even a minor risk is not acceptable in the light of treating otherwise healthy couples for infertility reasons. Therefore, it is of particular importance to investigate nontreated men's semen, because many individuals suffering from a mild form of COVID-19 might not even have associated their symptoms with infection by SARS-CoV-2.

Here, our study differs from the report of Song et al. who investigated disease-positive males confirmed by a positive oropharyngeal swap or anti–2019-nCoV serum antibodies, because all of their participants were treated with antiviral therapy that might have changed the presence or absence of SARS-CoV-2 in semen samples. This is also true for antibiotic treatment, corticosteroids, interferon, and immunoglobulins, which were administered in descending order. In addition, it is important to test semen even if the blood viral load is very low, because it was shown for HIV-1 that although the semen viral load is usually related to the viral load of the blood, in a minority of individuals the genital tract showed higher virus load than the blood. This suggests that viruses may have a local reservoir despite otherwise resilient immune control (16). Furthermore, because our investigation time frame of 32.7 days on average after the end of symptoms leaves the question unacknowledged whether there is a virus load in the semen in the early days of disease when symptoms are still present. Very recent data showed, however, that six out of 38 men with a positive nasopharyngeal swab who still had symptoms or stopped having symptoms 2–3 days before semen analysis presented with SARS-CoV-2 in the semen (17).

On another note, it is of interest that although it was described before in the literature that viral infections have a negative impact on semen parameters such as volume, number of spermatozoa, and motility we could not detect a negative influence of the SARS-CoV-2 infection regarding those sperm count parameters in recovered subjects with mild symptoms. However, patients facing a moderate course of disease and being in need of hospital care had reduced sperm quality (Table 2). On one hand, this could be an effect of the infection with SARS-CoV-2 in association with the severity of the illness or due to a transitory higher viral load. On the other hand, the impaired male fertility in this subgroup could be preexisting. Because these subjects were treated with lopinavir/ritonavir and hydroxychloroquine, an impact of these medications on sperm parameters is possible, although

unlikely because they were applied for only a few days. Moreover, there exists no evidence that lopinavir/ritonavir or hydroxychloroquine have an impact on male fertility (18, 19). In addition, it is noteworthy that in general modifications of the sperm count due to trauma, injury, or infection might be seen only after 3 months of time. Subsequently, another semen analysis after the aforementioned time would be desirable.

This study has certain limitations. First, we investigated a relative small sample size. Second, sperm analysis of tested individuals performed before the outbreak of the pandemic was not obtained, limiting the diagnosis of preexisting male infertility. Third, we analyzed only two patients with an active COVID-19 infection; it will be necessary to ascertain our findings in a lager sample size. Finally, our preliminary results lack any data about long-term effects of SARS-CoV-2 on male reproductive function.

CONCLUSION

In patients with mild symptoms, SARS-CoV-2 does not seem to have a short-term impact on male fertility regarding sperm characteristics according to WHO criteria. We found no evidence of SARS-CoV-2 shedding in semen of recovered men or men with an acute COVID-19 infection after a recovery time of 32.7 days on average.

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Evaluación de SARS-CoV-2 en semen humano – un estudio de cohorte.

Objetivo: Investigar la presencia de ARN viral en el semen humano de pacientes con síndrome respiratorio agudo severo por coronavirus 2 (SARS-CoV-2) y evaluar su presencia y relevancia en los parámetros seminales.

Diseño: Estudio piloto de cohorte.

Escenario: Hospital universitario.

Paciente(s): Treinta y cuatro hombres fueron distribuidos en: 1) pacientes en convalecencia (pacientes con infección por SARS-CoV-2 confirmada en hisopado faríngeo según reacción en cadena de la polimerasa de transcripción reversa [RT-PCR] o anticuerpos); 2) grupo control negativo (sin anticuerpos); y 3) pacientes con infección aguda (detección de SARS-CoV-2 en hisopado faríngeo).

Intervención: Se obtuvieron muestras de semen y sangre de cada individuo.

Medida(s) de Resultado Principal: El análisis de la calidad seminal de acuerdo a los parámetros de la Organización Mundial de la Salud. La detección de SARS-CoV-2 por RT-PCR en muestra de semen en fresco y después de la preparación por gradiente de densidad. La confirmación de anticuerpos de inmunoglobulina (Ig) A e IgG en sangre.

Resultado(s): Se obtuvieron dieciocho muestras de semen de hombres recuperados entre los 8-54 días después de la ausencia de síntomas, 14 de sujetos control, y 2 de pacientes con una infección activa por COVID-19. No se detectó ARN por RT-PCR en el semen, incluyendo las muestras de semen de los dos pacientes con infección aguda por COVID-19. Los sujetos con una infección moderada mostraron deterioro de la calidad seminal.

Conclusión(es): No es probable que una infección leve por COVID-19 afecte la función testicular y epididimaria, mientras que los parámetros seminales parecieron deteriorarse después de una infección moderada. El ARN de SARS-CoV-2 no pudo ser detectado en el semen de hombres COVID-19 positivos ya recuperados y con infección aguda. Esto sugiere que no hay transmisión viral durante el contacto sexual y las técnicas de reproducción asistida, aunque se necesita obtener más información.